44865734

Reviewer: Semalulu, Souleh , **Date** April 11, 2000

STUDY TYPE: Metabolism - *Supplementary* (with sub-chronic feeding) -Rat OPPTS 870.7485 [§85-1)]; OECD 417.

TEST MATERIAL (PURITY): [phenyl-UL-^{14}C] SZX 0722 [Iprovalicarb, Melody]

SYNONYMS: [phenyl-UL-^{14}C] SZX 0722, a 1:1 mixture of 2 diastereomers S,R [99.0%] and S,S. [98.2%].

CITATION: Knoell, H.-E., Anderson, C. (1997): [^{14}C]SZX 0722: Investigation of the biokinetic behaviour and the metabolism in the rat following subchronic feeding. Bayer AG, Report No. PF4322, 15/12/1997. MRID [not available]. Unpublished

SPONSOR: Tomen Agro Inc.

EXECUTIVE SUMMARY:

This rat biokinetics and metabolism study (MRID not available) was conducted using dose levels based on previous toxicology studies, in order to compare absorption, distribution, excretion and biotransformation between test groups with and without subchronic feeding with unlabelled SZX 0722 in the diet (2 days or 13 weeks of feeding), when given at doses of 500 and 20000 ppm. [Phenyl-UL-^{14}C]SZX 0722 was administered orally in 10 tests to 3 Wistar rats/sex. The single radioactive gavage dose was 2 mg/kg bw (in test number 1-8), and at 4 mg/kg bw in the whole-body autoradiography (test nos 9-12). In tests 11-12, [valine-1-^{14}C]SZX 0722 was used instead of the phenyl-UL-labelled compound.

The absorption and excretion data from this study did not differ qualitatively from those determined in the standard metabolism study. The plasma curves were comparable between all groups, although the maximum equivalent concentration in plasma was slightly reduced after sub-chronic feeding (0.17-0.21 µg/g versus 0.19-0.29 µg/g). The maximum was reached about 1 h after administration. Female rats had lower concentrations of total radioactivity in plasma than males after feeding at 20 000 ppm unlabelled SZX 0722. The plasma radioactivity concentrations decreased quickly after the maximum levels were achieved. Plasma levels were about 50% of the maximum 3-4 h post-application, falling then to about 0.001-0.003 µg/g after 48 h (72 h in the high-dose female rat group without sub-chronic feeding). Whole-body autoradiography 4 h post-administration confirmed the rapid absorption and distribution of radioactivity.The radioactivity was mainly found in liver, stomach, kidney and small intestine. Lower levels of radioactivity were found in the heart and lung, with virtually no radioactivity being found in the brain. Up to 99% or more of the administered dose (AD) was excreted via urine and faeces within 72 h following administration of the radioalabelled material. The major route of excretion was faecal (about 70%) for male rats. About equal proportions were excreted through the faecal and urinary route in females. No effect of dose or sub-chronic feeding on these parameters was evident.

At the sacrifice (72 h post-administration), the radioactivity remaining in the body (outside the gastrointestinal tract) was extremely low, (less than 0.25% AD) and was not effected by sub-chronic feeding. Whole-body autoradiography confirmed the rapid excretion of radioactivity from the rat body. At 48 h post-administration, the radioactivity was mainly localized in small intestine, liver and large intestine and, to a small extent, in the kidney cortex. The quick depletion of radioactivity from all organs and tissues except liver and the gastrointestinal tract indicates that there was no effect of sub-chronic feeding on distribution and excretion. The situation was different in the tests using the valine-1-labelled compound instead of the phenyl-UL-label. After 48 h, higher levels of radioactivity were detected radiographically in most tissues and organs. This reflects the fact that the enzymatic cleavage of the amide bond in SZX 0722 results in production of fragments such as labelled valine, which can be channelled into the endogenous metabolism and eventually incorporated into body proteins.

Based on the results from the previous metabolism experiment, the quantitative analysis of the excreta was focussed on parent compound SZX 0722, the main metabolite SZX 0722 carboxylic acid (*M03*), the minor metabolites 4-(1-hydroxyethyl)-benzoic acid (*M20*) and PMPA (p-methylphenethylamine, *M10*) as representative of the metabolic pattern. No effort was made to quantify other trace components. In addition, the ratios of isomers S,S versus S,R of the parent compound and the major metabolite were determined. The content of major metabolite (*M03*), about 80-90 % of total urinary radioactivity was not affected by chronic feeding. However the isomer ratio was shifted in favour of S,R isomer when urinary excretion at the pretreatment level of 500 ppm was compared to 20,000 ppm. This effect was more prominent in males than in females. The low dose group males showed an isomer ratio of 38:1, whereas the high dose males had a ratio of 5:1. The corresponding low and high dose isomer ratios for females was 2.7:1 and 1.8:1 respectively. Females excreted a higher amount of S,R isomer, so the shift in favour of S,R was less pronounced. This sex dependent isomer ratio was also noted in the main metabolism study. The proportion of minor metabolites (*M20*) was neither affected by chronic feeding, nor by the dose. The amount of (*M20*) excreted by females was consistently double or greater than the amount excreted by males, reflecting the sex dependent route of excretion. Very low amount of the minor metabolite *(M10)* were found in urine after hydrolysis, but there was a 4-40 fold increase in this metabolite with the increase in dosing.

COMPLIANCE: Signed and dated GLP, Quality Assurance, and Data Confidentiality statements were provided.

I. MATERIALS AND METHODS

A. MATERIALS:

1. Test Compound:

SZX 0722 = 1:1 mixture of 2 diastereomers S,R (99.3%) and S,S, (99.5%).

Radiolabelled Test Materials:	[Phenyl-U-^{14}C] SZX 0722
Radiochemical purity	98 % determined by HPLC, TLC , 50:50 diastereomer ratio.
Specific Activity	138.0 μCi/mg
Lot/Batch #:	S,R batch # KML 2320
	[Valine-1-^{14}C] SZX 0722
Radiochemical purity	99% determined by HPLC , TLC, 52:48 diastereomer ratio.
Specific Activity	103.5 μCi/mg
Lot/Batch #:	KML 2427 + KML 2475
CAS #:	140923-25-7
Non-Radiolabelled Test Material:	SZX 0722
Description:	1:1 mixture of diastereomers, colourless powder, stable at ambient or refrigeration temperatures
Lot/Batch #:	S,S batch no 960708ELB02, S,R batch no 960515ELB03, S.R and S,S mixture (batch no 960515ELB03)
Purity:	99.2-99.9 % a.i. determined by HPLC, or TLC
Contaminants:	
CAS #:	140923-25-7
Structure, including location of label	

2. Vehicle and/or positive control: 0.5% aqueous tragacanth, Lot/Batch #; Purity not provided.

3 Test animals:

Species:	Rat	
Strain:	Wistar Hsd/Win:WU, male and female	
Age/weight at study initiation:	400 g males, 200g females	
Source:	Winkelmann Versuchsteierzucht GmbH & Co. KG 33178 Borchen, FRG	
Housing:	Singly in plastic Type II Macrolon cages, or special metabolism cages	
Diet:	Standard food (Altromin 1324 Fa. Altrogge, 32791, FRG), spiked with either 500 or 20,000 ppm unlabeled SZX 0722, fed *ad libitum*.	
Water:	Tap water, *ad libitum*	
Environmental conditions:	**Temperature:**	18 - 26°C
	Humidity:	27 - 81 %
	Air changes:	3000ml/min
	Photoperiod:	12 hrs dark/ 12 hrs light
Acclimation period:	7 days	

4. Preparation of dosing solutions:

Unlabelled SZX 0722 (4.81-4.89 mg was used for the dilution of the radiolabelled substance, and as reference material. The radiolabelled test compound (3.2 mg ^{14}C-SZX 0722) was dissolved in acetonitrile, dried with nitrogen gas, added to 5 ml ethanol, air dried and dissolved in 20 ml, 0.5% aqueous tragacanth solution. Preparation of homogeneous suspension required digestion of test substance with tragacath solution at 70°C for 30 minutes using supersonics, followed by overnight stirring at the same temperature. Dose volumes 1.1-2.0 ml/animal were calculated based on animal body weight

B. STUDY DESIGN AND METHODS:

1. Group Arrangements

Animals were assigned to the test groups noted in Table 1 using randomization by lot.

2. Dosing and sample collection:

a. Pharmacokinetic studies

Test numbers 1-8 were used for biokinetic studies and radioactivity balance studies. Blood was collected at 0.08, 0.17, 0.33, 0.67, 1., 1.5, 2, 3, 4, 6, 8, 24, 32, 48, 56, 56, and 72h and used for plotting of time course of plasma radioactivity concentration. Urine and faeces extracts were analysed for active ingredients and metabolites, by TLC, HPLC, and or GC. In control experiment (Test No.13) unlabelled SZX0722 was used. Test numbers 9,10,11 and 12 were the whole body radiography experiments.

b. Metabolite characterization studies

After dosing with radiolabelled test compound, animals were kept in individual metabolism cages to allow separate and quantitative collection of urine and faeces. Urine was collected separately for each animal, in the intervals 0-4, 4-8, 8-24, 24-48 h in all test groups, and in addition the 48-72 h interval for tests 1-8. Faeces were collected separately for each animal, in the intervals 0-24, 24-48, 8-24, 24-48h in all test groups, and in addition the 48-72h interval for tests 1-8.

Animals were sacrificed following carbon dioxide anaesthesia at 72h in test numbers1-8, and at 48h in test numbers 9-12. They were exsanguinated, and blood, organs and tissues were collected. The blood was separated into plasma and erythrocytes. The carcass and tissues were weighed immediately after dissection, lyophilised then homogenised before determination of radioactivity following combustion.

3. Statistics:
Statistical computation were made using a dedicated software package. Values were checked for outliers by the NALIMOV test, followed by calculation of arithmetic means.

TABLE 1: Dosing groups for pharmacokinetic studies for SZX 0722

Test No.	Dose, labelled material (mg/kg bw)	Number of animals	SZX 0722 in feed		Radiolabeled test compound	Test objective
			time	ppm		
1	2	3m	2 days	500	[phenyl-UL ^{14}]C-SZX 0722	excretion in urine and faeces (72h)
2	2	3f	2 days	500	[phenyl-UL ^{14}]C-SZX 0722	excretion in urine and faeces (72h)
3	2	3m	2 days	20000	[phenyl-UL ^{14}]C-SZX 0722	excretion in urine and faeces (72h)
4	2	3f	2 days	20000	[phenyl-UL ^{14}]C-SZX 0722	excretion in urine and faeces (72h)
5	2	3m	13 wk	500	[phenyl-UL ^{14}]C-SZX 0722	plasma and tissue concentration
6	2	3f	13 wk	500	[phenyl-UL ^{14}]C-SZX 0722	plasma and tissue concentration
7	2	3m	13 wk	20000	[phenyl-UL ^{14}]C-SZX 0722	plasma and tissue concentration
8	2	3f	13 wk	20000	[phenyl-UL ^{14}]C-SZX 0722	plasma and tissue concentration
9	4	3m	13 wk	20000	[phenyl-UL ^{14}]C-SZX 0722	urinary and faecal excretion (48h) + autoradiography
10	4	3f	13 wk	20000	[phenyl-UL ^{14}]C-SZX 0722	urinary and faecal excretion (48h) + autoradiography
11	4	3m	13 wk	20000	Valine-1 ^{14}]C-SZX 0722	urinary and faecal excretion (48h) + autoradiography
12	4	3f	13 wk	20000	Valine-1 ^{14}]C-SZX 0722	urinary and faecal excretion (48h) + autoradiography
13	4	1/sex	13 wk	20000	SZX 0722 unlabeled	Control

m = male; f= female

II. RESULTS

A. Pharmacokinetic Studies:

1. Preliminary experiment Not applicable.

2. Absorption

The dose normalised plasma concentration of radiolabelled test material with time is presented in Table2. Phenyl-UL ^{14}C]SZX 0722 was absorbed rapidly from the gastrointestinal tract in all dose groups after oral administration. The plasma curves showed that absorption started immediately after dosing. Maximum plasma concentrations were reached at about 60 minutes post dosing in all dose groups. Maximum equivalent concentrations were lower in rats after sub-chronic feeding compared to rats which received SZX 0722 in the feed for 2 days only. Females had lower concentrations of total radioactivity in plasma than males after feeding with 20000 ppm a.i.

3. Tissue distribution

In all dose groups, plasma curves showed a quick decrease in radioactivity concentration when the maximum levels were attained, which was attributable to fast distribution and excretion. About 3-4 hours after dosing, plasma levels were 50% of the maximum levels attained, and fell to about 0.001-0.0003 μg/g in all test groups except the 20 000 ppm test group (group number 4) where plasma levels dropped to 0.003 μg/g after 72 hours. Whole-body autoradiography findings mirrored plasma curve findings, manifesting rapid distribution, and excretion. Even though the dose used in whole-body autoradiography experiments was set at 4 mg/kg bw in order to improve detection limits, the results were still comparable to those of the main test dosed at 2 mg/kg bw. Following oral dosing, the highest radiographic concentrations were observable in the liver, stomach, kidney and small intestines after 4 hours. Lower levels were present in the heart, lungs, salivary glands and infraorbital glands. Very low levels were in blood, muscle, and testes, and even lower levels were present in brain, spinal marrow and lower part of the large intestines. After 48h highest concentrations were in the small and large intestines followed by the liver. The intensity of radioactivity in the kidneys and stomach wall was just above background. The distribution was similar in both sexes. The high concentrations present in the liver and small intestines indicate the possibility of enterohepatic circulation of the compound derived radioactivity. The rapid excretion of Phenyl-UL ^{14}C]SZX 0722 led to quick depletion within 48h, from all the organs except the liver and kidney. This finding shows that sub-chronic feeding had no influence on tissue levels, and parallels results from the other main metabolism study.

In animals dosed with [valine-1-^{14}]C-SZX 0722, the highest radiographic concentrations were in the liver, stomach, kidney and small intestines, salivary glands and infraorbital glands, four hours after oral dosing. Lower levels were present in the heart, lungs, heart and testes. Very low levels were in brain and bone marrow, and none in the eye. After 48 hours, high levels of radioactivity were present in most tissues and organs. Highest concentrations were in the intestines, liver, femur, salivary glands and bone marrow. Lower concentrations were present in the kidney, lungs, heart, adrenals, testis. Even lower levels were detectable in muscle, brain and spinal marrow. The distribution was similar in both sexes.

The widespread high intensity of radio label observed in many tissues of animals treated with [valine-1-^{14}]C- labelled test compound indicate that enzymatic cleavage of the amide bond of SZX 0722 resulted in fragments like valine which were channelled into the endogenous metabolism and eventually incorporated into body protein.

Excretion

Recovery of radioactivity in tissues and excreta 72h after dosing radiolabelled test material is presented in Table 3. In all dose groups, 99.2% or more of the administered dose (AD) was excreted through urine and faeces within 72h. The major route of excretion was faecal in males (2:1 faecal vs urinary), and equal proportions (1:1) were excreted through faeces and urine in females. The excretion rates, and routes did not vary with dose or pre-treatment with non-radiollabeled compound. The radioactivity remaining in the body (excluding GIT) after 72h was less than 0.25% AD in all test groups. Pre-treatment resulted in approximately a doubling of the amount of residue [(0.13% and 0.08% AD in males and females respectively dosed at 500 ppm for 2 days, vs (0.25% and 0.19% AD in animals pre-dosed at 500 ppm for 13 wks), and (0.09/0.07% AD in males/females pre-dosed at 20000 ppm for 2 days vs 0.13/0.1% AD in males and females pre-dosed at 20,000 ppm 13 weeks]. In the rest of the carcass (excluding the GIT and major organs) less than 0.22% AD was detectable 48h after dosing, with sub-chronic feeding having minimal effect on these residues, regardless of dose levels in feed.

B. <u>Metabolite characterization studies</u>:

Metabolism

The total balance of metabolites in excreta is presented in Tables 4. Quantitative determination of metabolites in composite in urine and faeces are presented in Tables 5 and 6, respectively. In females, a larger proportion of parent compound was excreted following pretreatment with 20,000 ppm (17.1% AD compared to pretreatment with 500 ppm SZX 0722 (4.3% AD) , but no effect of sub-chronic feeding was evident for males. The metabolites identified in urine and faecal extracts amounted to 91% or more of the total radioactivity in the respective matrixes. The main metabolite in urine was BNF 5571B (M03) the carboxylic acid metabolite of the parent compound. Unchanged parent compound accounted for < 1% radioactivity. All other known components were in the range 0.4 to 4% with 4-(1-hydroxyyl) benzoic acid [ANC 0150 (M20)] having the highest peak by HPLC. The metabolite p-methylphenethylamine [PMPA, (M10)] was found as a conjugate, accounting for 0.4% renally excreted radioactivity. In faeces, the major radioactive components were BNF 5571B and the parent compound SZX 0722. There was a clear effect of the dose given in the diet on the proportions of these components. From 2 days to 13 weeks at 500 ppm, the ratio of BNF 5571B:SZX 0722 shifted from 94:1 to 40:1in males and from 25:1 to 19:1i n females. From 2 days to 13 weeks at 20,000 ppm, this ratio shifted from 0.6:1 to 0.4:1 in males and from 1.5:1 to 0.2:1 in females. However, there was no effect of sub-chronic feeding on the ratio of SZX 0722 isomers S,S : S,R. The basic metabolic pattern remained very similar to that seen in the previous experiment, with only minor changes being noted. In composite urine, M03 and M20 were determined by HPLC. The content of M03 (about 80-90% of the total renal radioactivity) was not affected by subchronic feeding. However, the isomer ratio S,S:S,R was shifted in favour of the S,R isomer when comparing the tests with 500 and 20 000 ppm of SZX 0722 in the feed. This effect was much more distinct in male rats than in females. The low-dose males exhibited an isomer ratio of about 38:1, whereas the high dose group had a ratio of about 5:1. The corresponding ratios for female rats were 2.7:1 and 1.8:1. Female rats excreted a higher amount of the S,R isomer in general, so that the shift in favour of the S,R isomer was less pronounced. This sex dependency of the isomer ratio was noted earlier (cf. 5.1.1). The percentage of 4-(1-hydroxyethyl)-benzoic acid (*M20*) was not affected by sub-chronic feeding or by the dose. The amount of M20 excreted by female rats was always twice the amount excreted by males (in % of the radioactive dose), and thus simply reflects the sex-dependent routes of excretion.

Very low amounts of PMPA (p-methylphenethylamine, *M10*) were found in composite urine after acid hydrolysis (<1% of the dose). A 4 to >10-fold increase in the amounts of PMPA in urine was evident upon increasing the dose in the diet (cf. table 5.1.2.c). Unchanged parent compound occurred only in very

minute quantities in the urine (always <1%). The total radioactivity recovered (TRR) from composite faeces by extraction was high, amounting at least 92.5%. The acetonitrile extracts accounted for 11-47% and the water-extracts for 47-84% of faecal TRR.

In the acetonitrile extracts of composite faeces, the parent compound SZX 0722 and its major metabolite SZX 0722 carboxylic acid (*M03*) were present in varying ratios. Feeding with 20 000 ppm of SZX 0722 in the diet resulted in significantly higher proportions of parent compound (SZX 0722) in the extracts (4-14% of the dose) than feeding with 500 ppm (<1%). In addition, an effect of sub-chronic feeding was evident in female rats, with about 4 and 17% unchanged SZX 072 in the extracts after 2 days and 13 weeks of pretreatment, respectively. This distinct shift in favour of parent compound did not significantly affect the isomer ratio of SZX 0722; the isomer ratio of M03 was affected slightly. In the aqueous extracts of composite faeces, M03 was the predominant metabolite at ≥90% of the analyzed radioactivity. Neither the percentage of M03 nor its isomer ratio were significantly effected by of sub-chronic feeding. The diastereomer ratio of the main metabolite SZX 0722 carboxylic acid (*M03*) in the total excreta was very close to 1:1 in all tests.

TABLE 2: Phenyl-UL ^{14}C]SZX 0722: mean relative concentration P (μg/g) in plasma at different time intervals following single oral dosing at 2 mg/kg bw.[a] (expressed as dose normalised concentration).

Test No.	1	2	3	4	5	6	7	8
Sex	m	f	m	f	m	f	m	f
Preteatment Amount (ppm)	2 days 500	2 days 500	2 days 20,000	2 days 20,000	13 wk 500	13 wk 500	13 wk 20,000	13 wk 20,000
Time post-dose (h)								
0.08	0.05	0.05	0.014	0.016	0.027	0.039	0.012	0.019
0.17	0.08	0.11	0.068	0.049	0.079	0.077	0.063	0.05
0.33	0.11	0.11	0.112	0.064	0.083	0.093	0.018	0.066
0.67	0.11	0.12	0.146	0.085	0.099	0.095	0.095	0.077
1	0.14	0.12	0.148	0.101	0.106	0.109	0.106	0.08
1.5	0.13	0.11	0.13	0.098	0.104	0.097	0.096	0.07
2	0.11	0.09	0.11	0.09	0.091	0.08	0.078	0.065
3	0.09	0.06	0.071	0.065	0.074	0.056	0.055	0.039
4	0.07	0.03	0.055	0.048	0.058	0.036	0.04	0.031
6	0.04	0.02	0.035	0.037	0.039	0.028	0.026	0.022
8	0.03	0.02	0.024	0.02	0.028	0.021	0.017	0.018
24	0.01	0.0045	0.004	0.015	0.01	0.01	0	0.005
32	0.004	0.0028	0.003	0.007	0	0	0	0.002
48	0	0.001	0.001	0.006	0	0	0	0.001
56	0	0.001	0	0.002	0	0	0	0.001
72	0	0.001	0	0.002	0	0	0	0

a Data extracted from pgs (36) of the study; m = male, f = female

TABLE 3: Recovery of radioactivity in tissues and excreta 72h after dosing with Phenyl-UL ^{14}C-SZX 0722 [a] (expressed as a % of dose).

Test No.	1	2	3	4	5	6	7	8
Sex	m	f	m	f	m	f	m	f
pretreatment amount (ppm)	2 days 500	2 days 500	2 days 20,000	2 days 20,000	13 wk 500	13 wk 500	13 wk 20,000	13 wk 20,000
urine	32.63	55.64	30.48	53.2	32.2	52.6	29.6	49
faeces	67.02	45.26	73.97	50.1	70.1	48.7	72.1	50.2
skin	-	-	-	-	0.038	0.03	-	-
Sum organs	0.13	0.08	0.09	0.068	0.216	0.152	0.117	0.098
Body less GIT	0.13	0.08	0.09	0.068	0.254	0.186	0.129	0.098
GIT	0.22	0.03	0.14	0.084	0.588	0.195	0.21	0.307
Total body	0.35	0.1	0.23	0.153	0.842	0.381	0.339	0.405
Balance	99.98	102.4	104.7	103.5	103.1	101.8	102	99.6

a Data extracted from pgs (41) of the study report

TABLE 4. Total balance of excreted metabolites (expressed as percent of the administered dose) in urine and faeces of rats dosed with Phenyl-UL-^{14}C-labelled test material.

Test No.	1	2	3	4	5	6	7	8
Sex	m	f	m	f	m	f	m	f
pretreatment amount (ppm)	2 days 500	2 days 500	2 days 20,000	2 days 20,000	13 wk 500	13 wk 500	13 wk 20,000	13 wk 20,000
Metabolites								
SZX 0722	0.09*	0.23	13.7	4.28	0.18	0.24	13.33	17.09
Isomer S,S	0.05	0.12	8.88	2.78	0.08	0.24	13.33	17.09
Isomer S,R	0.04	0.11	4.82	1.51	0.11	0.15	5.66	6.99
BNF 5571B	84.97*	89.89	77.33	82.92	86.25	83.8	74.63	71.56
'Isomer S,S	40.9	43.85	35.5	41.43	42.63	39.75	33.85	33.04
'Isomer S,R	44.08	46.04	41.82	41.47	43.6	44.05	40.74	38.51
ANC 0150	0.96*	2.05	1.15	2.02	0.81	2.25	0.8	1.68
PMPA**	0.1*	0.05	0.36	0.29	0.12	0.03	0.61	0.87
Total rest***	9.24	7.51	7.87	9.28	9.43	9.24	9.07	4.68
Total	95.36*	99.73	100.41	98.79	96.79	95.56	98.44	95.88
% of dose (urine + faeces extracts)	95.48	100.17	100.4	98.78	96.78	95.58	98.44	95.88
Loss	0.12	0.44	-0.01	-0.01	-0.01	0.02	0	0

Data extracted from page 45 of the study report.

* In tests 3 and 8 the amount of SZX 0772 from water extracts of faeces was added with same isomer ratio as for ACN extracts.

** Data subject to a variation of > 100% due to limitations of the method.

*** Total rest" = rest (urine–PMPA) + rest faeces (ACN) + rest faeces (H_2O)

TABLE 5. Quantitative determination of metabolites in composite urine (as percent administered dose).

Test No.:	1	2	3	4	5	6	7	8	9	11
sex	m	f	m	f	m	f	m	f	m	m
pretreatment	2 d	2 d	2 d	2 d	13 wk	13 wk	13 wk	13 wk	13 wk	13 wk
ppm	500	500	20000	20000	500	500	20000	20000	20000	20000
	% of Dose									
M20	0.96	2.05	1.15	2.02	0.81	2.25	0.80	1.68	0.65	n.d.*
M03	24.91	47.73	24.01	44.51	25.30	40.35	22.06	42.73	16.58	13.41
isomer S,S	24.37	34.86	19.36	30.18	24.52	29.55	18.86	25.24	13.91	11.33
isomer S,R	0.56	12.87	4.65	14.32	0.77	10.80	3.18	17.48	2.67	2.09
rest**	5.13	4.23	4.34	6.27	4.20	6.90	5.04	1.59	3.77	1.62
total	31.12	54.45	29.49	52.83	30.29	49.51	27.90	46.00	20.96	15.03

* n.d. = not detectable (due to valine-1 label) ** "rest" used for determination of PMPA in urine (cf. table 5.1.2.c)
M20 = 4-(1-hydroxyethyl)-benzoic acid. M03 = SZX 0722 carboxylic acid

TABLE 6. Quantitative determination of metabolites in composite faeces (as percent administered dose).

Test No.:	1	2	3	4	5	6	7	8	9	11
sex	m	f	m	f	m	f	M	f	m	m
pretreatment	2 d	2 d	2 d	2 d	13 wk	13 wk	13 wk	13 wk	13 wk	13 wk
ppm	500	500	20000	20000	500	500	20000	20000	20000	20000
	% of Dose									
Acetonitrile extract										
a.s.	0.09	0.23	13.49	4.28	0.18	0.24	13.33	16.79	9.50	15.07
isomer S,S	0.05	0.12	8.74	2.78	0.08	0.09	7.67	9.92		
isomer S,R	0.04	0.11	4.75	1.51	0.11	0.15	5.66	6.87		
M03	8.46	5.83	8.45	6.32	7.27	4.60	5.86	3.68	3.71	8.24
isomer S,S	2.15	1.21	2.39	1.60	1.93	0.99	1.63	0.99	0.93	2.73
isomer S,R	6.30	4.62	6.06	4.71	5.34	3.61	4.22	2.68	2.79	5.50
rest	0.45	0.50	0.96	0.54	0.18	0.24	0.21	1.46	0.23	0.61
total	9.00	6.56	22.90	11.15	7.64	5.08	19.40	21.92	13.45	23.91
Aqueous extract										
a.s.	-	-	0.21	-	-	-	-	0.30	-	-
M03	51.60	36.33	44.87	32.09	53.68	38.85	46.71	25.15	25.93	48.86
isomer S,S	14.38	7.78	13.75	9.65	16.18	9.21	13.36	6.80	6.73	17.07
isomer S,R	37.22	28.55	31.11	22.44	37.49	29.64	33.34	18.35	19.19	31.79
rest	3.76	2.83	2.93	2.71	5.17	2.13	4.43	2.50	1.58	2.12
total	55.36	39.16	48.01	34.80	58.85	40.99	51.14	27.96	27.51	50.98

a.s. = active substance (SZX 0722)
M03 = SZX 0722 carboxylic acid

III. DISCUSSION

A. Investigators' conclusions

The rates of absorption, distribution, biotransformation and excretion of radioactivity, (with and without sub-chronic feeding at two dose levels with unlabelled test material) were compared in rats of both sexes following oral dosing with phenyl-UL-[^{14}C]SZX 0722 or Valine-1-[^{14}C]SZX0722. After 2 days or 13 weeks of feeding with SZX 0722 at dose levels of 500 or 20 000 ppm in the diet, radioactively labelled compound was administered once to 12 groups of male or female rats. The radioactivity in plasma and excreta were measured, and quantitative analysis of the excreta for four defined components (representative of the metabolic pattern) was performed. In addition, autoradiographic evaluations were conducted. The objective was to examine eventual differences in metabolic or biokinetic behaviour attributable to dose or sub-chronic feeding. The absorption and excretion data from this study did not differ qualitatively to any major extent from those determined in the standard study. An average of 99% or more of the administered dose was excreted via urine and faeces within 72 h of the radioactive dose. The major route of excretion was faecal for male rats (about 70%) and about equally through the faecal and renal route for female animals. No effect of dose or sub-chronic feeding on these parameters was evident. The concentration maximum in plasma was reached approximately 1 hour after dosing. The plasma curves were similar in all groups, although the maximum equivalent concentration in plasma was slightly reduced after sub-chronic feeding (0.17-0.21 µg/g versus 0.19-0.29 µg/g). High-dose female rats had lower concentrations of total radioactivity in plasma than males.

No major effects on the general metabolic pattern of SZX 0722 were seen in this experiment as compared to the standard metabolism study. The primary compound detected in all excreta was SZX 0722 carboxylic acid (*M03*). The isomer ratio S,S:S,R was shifted in favour of the S,R isomer in the urine of rats which received 20 000 ppm in the feed as compared to those receiving 500 ppm. The effect was much more distinct in male rats than in females; a very minimal effect was also observed in the faeces. The higher dose groups also exhibited higher proportions of unchanged parent compound in the faeces. In addition, higher amounts of SZX 0722 were evident in the faeces of female rats after sub-chronic feeding. There was no shift in the isomer ratio of the excreted unchanged SZX 0722. Very low amounts of PMPA (*M10*) were found in the urine (<1% of the dose). A 4 to >10-fold increase in the amounts of the metabolite was apparent in the high-dose groups.

B: Reviewer comments:

This rat biokinetics and metabolism study was conducted using dose levels based on previous toxicology studies, in order to compare absorption, distribution, excretion and biotransformation between test groups with and without subchronic feeding with unlabelled SZX 0722 in the diet (2 days or 13 weeks of feeding), when given at doses of 500 and 20 000 ppm. [Phenyl-UL-[^{14}C]SZX 0722 was administered orally in 10 tests to 3 Wistar rats/sex. The single radioactive gavage dose was 2 mg/kg bw (in test number 1-8), and at 4 mg/kg bw in the whole-body autoradiography (test nos 9-12). In tests 11-12, [valine-1-[^{14}C]SZX 0722 was used instead of the phenyl-UL-labelled compound.

The absorption and excretion data from this study did not differ qualitatively from those determined in the standard study. The plasma curves were comparable between all groups, although the maximum equivalent concentration in plasma was slightly reduced after sub-chronic feeding (0.17-0.21 µg/g, v.s. 0.19-0.29 µg/g). The maximum was generally reached about 1 h after administration. Female rats had lower concentrations of total radioactivity in plasma than males after feeding with 20000 ppm of a.i. The

plasma radioactivity concentrations decreased fairly quickly after the maximum levels were achieved. Plasma levels were about 50% of the maximum 3-4 h post-application, falling then to about 0.001-0.003 µg/g after 48 h (72 h in the high-dose females without sub-chronic feeding). Whole-body autoradiography 4 h post-administration confirmed the rapid absorption and distribution of radioactivity. The radioactivity was mainly found in liver, stomach, kidney and small intestine. Lower levels of radioactivity were found in the heart and lung, with virtually no radioactivity being found in the brain. Up to 99% or more of the administered dose (AD) was excreted via urine and faeces within 72 h following administration of the radioalabelled material. The major route of excretion was faecal (about 70%) for male rats. About equal proportions were excreted through the faecal and urinary route in females. No effect of dose or sub-chronic feeding on these parameters was evident.

At sacrifice (72 h post-administration), the radioactivity remaining in the body excluding the gastrointestinal tract was extremely low, (less than 0.25% AD) and not effected by sub-chronic feeding. Whole-body autoradiography confirmed the rapid excretion of radioactivity from the rat body. At 48 h post-administration, the radioactivity was mainly localized in small intestine, liver and large intestine and, to a small extent, in the kidney cortex. The quick depletion of radioactivity from all organs and tissues except liver and the gastrointestinal tract indicates that there was no effect of subchronic feeding on distribution and excretion. The situation was different in the tests using the valine-1-labelled compound instead of the phenyl-UL-label. After 48 h, higher levels of radioactivity were detected radiographically in most tissues and organs. This reflects the fact that the enzymatic cleavage of the amide bond in SZX 0722 results in production of fragments such as labelled valine, which can be channelled into the endogenous metabolism and eventually incorporated into body proteins. Based on the results from the previous metabolism experiment, the quantitative analysis of the excreta was focussed on parent compound SZX 0722, the main metabolite SZX 0722 carboxylic acid (*M03*), the minor metabolites 4-(1-hydroxyethyl)-benzoic acid (*M20*) and PMPA (p-methylphenethylamine, *M10*) as representative of the metabolic pattern. No effort was made to quantify other trace components. In addition, the ratios of isomers S,S versus S,R of the parent compound and the major metabolite were determined. The concentration of SZX 0722 carboxylic acid (*M03*) and 4-(1-hydroxyethyl)-benzoic acid (*M20*) were determined by HPLC in composite urine.

The content of major metabolite (*M03*), about 80-90 % of total urinary radioactivity was not affected by sub-chronic feeding. However the isomer ratio was shifted in favour of S,R isomer when urinary excretion at the pretreatment level of 500 ppm was compared to 20,000 ppm. This effect was more prominent in males than in females. The low dose group males showed an isomer ratio of 38:1, whereas the high dose males had a ratio of 5:1. The corresponding low and high dose isomer ratios for females was 2.7:1 and 1.8:1 respectively. Females excreted a higher amount of S,R isomer, so the shift in favour of S,R was less pronounced. This sex dependent isomer ratio was also noted in the main metabolism study. The proportion of minor metabolites (*M20*) was neither affected by sub-chronic feeding, nor by the dose. The amount of (*M20*) excreted by females as a percentage of the administered dose was consistently double or greater than the amount excreted by males, reflecting the sex dependent route of excretion. Very low amount of the minor metabolite *(M10)* were found in urine after hydrolysis, but there was a 4-40 fold increase in this metabolite with the increase in dosing.

C. Study deficiencies:

This study is acceptable as supplementary. The number of animals per test group were small (1-3/group). The findings complements the results of the main metabolism study conducted with the appropriate sample sizes.